

REMARKS

After entry of this amendment, claims 1-15 are pending. Applicants hereby request non-entry of the previously filed unentered amendments and request that the amendments above be entered. Claims 1 and 13 have been amended without disclaimer or prejudice and find support *inter alia* in the original claims and in the specification, for example, at page 7, lines 39-42. Claim 15 has been amended to correct an obvious typographic error. Support is found *inter alia* in the original claim. No new matter has been added.

Applicants enclose herewith a Request for Continued Examination requesting entry of the above claim amendment and consideration of the present remarks. The above claim amendment and following remarks address the rejection in the Final Office Action dated April 28, 2009 and also the comments in the Advisory Action mailed August 6, 2009.

Rejections under 35 U.S.C. 103(a)

Wilms in view of Moralejo

Claims 1-11 and 13-15 stand rejected as being obvious under 35 U.S.C. § 103(a) over Wilms *et al.* (2001; hereinafter “Wilms”) in view of Moralejo *et al.* (1993; hereinafter “Moralejo”). Applicants respectfully disagree. However, in order to expedite prosecution, the claims have been amended without disclaimer or prejudice and recite the concentration of the L-rhamnose in the medium. Applicants respectfully urge reconsideration of the rejection for the reasons already of record and additionally for the following reasons.

In response to the arguments presented in the Amendment and Reply of December 22, 2008, the Examiner alleges that features not recited in the claims were relied on; for example, specific growth parameters (see Office Action dated April 28, 2009, page 10). In light of the present amendment, Applicants request reconsideration.

Neither Wilms nor Moralejo teach or suggest a concentration of L-rhamnose in the medium from 0.01 g/l to 0.5 g/l as now claimed. Because Wilms and Moralejo, alone or in combination, do not teach or suggest all the claim limitations, a *prima facie* case of obviousness has not been established. See *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993) (holding that the Examiner bears the initial burden of establishing *prima facie* obviousness); *In re Lowry*, 32 F.3d 1579, 1582, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994) (to

support a *prima facie* conclusion of obviousness, the prior art must disclose or suggest all the limitations of the claimed invention).

Moreover, it is well established that under 35 U.S.C. § 103 the Examiner must consider the reference in its entirety, *i.e.* as a whole, including portions that teach away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); see also *KSR*, 127 S. Ct. at 1740; MPEP § 2141.03 (VI). It is improper to combine references where the references teach away from their combination. See MPEP § 2145 (X)(D)(2) (citing *In re Grasselli*, 713 F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983)).

The present invention relates to an improved method for expressing nucleic acids in prokaryotic cells using the rhaBAD promoter where surprisingly small quantities of L-rhamnose give high expression levels. (Specification, page 5, lines 1-4). In contrast to the low amount of L-rhamnose needed as claimed, Wilms describes that a substantially higher concentration of rhamnose has to be used (Wilms, page 100, left column and Figure 6) (see detailed explanations in the Amendment and Reply of December 22, 2008). Wilms further teaches that “[a]t the concentration of 0.5 g L⁻¹, the rhamnose was almost completely taken up from the cells [...]” and that only with the addition of 2 g/L rhamnose was it possible to maintain induction over a prolonged period: “A rhamnose concentration of 2 g L⁻¹ seemed to be optimal.” (Wilms, page 100, left column, and Figure 6). Consequently, a sufficient expression of a gene controlled according to Wilms is not ensured at a concentration of less than 2 g/L in the fermenter. Thus, Wilms leads away from using the small concentration as now claimed.

Moreover as explained in detail in the Amendment and Reply of December 22, 2008, because Wilms specifically teaches preference for the mutation in the RhaB gene,¹ because the expression system of Wilms takes advantage of the strictly regulated rhaBAD promoter,² and because, as stated in the International Preliminary Examination Report (IPER), the RhaB negative-strain is recommended especially for fermentations carried out as batch fed processes,

¹ This preference is because phosphorylation of L-rhamnulose by the RhaB gene is the first irreversible step in the degradation of L-rhamnose to dihydroxyacetone phosphate and L-lactaldehyde. (Wilms, page 98, left column, lines 4-8).

² Wilms, page 95-96, right column, last sentence.

Wilms disregards using any other enzyme as a potential target. Therefore, Wilms leads away from the substitution suggestion by the Examiner.

The Examiner also alleges that one skilled in the art would be motivated to use the method of Wilms and modify this method by inactivating the RhaA gene because Moralejo allegedly teaches that inactivation of the RhaA gene would be expected to block any catabolism of L-rhamnose in the cell. The Examiner then concludes that this would greatly reduce the amount of expensive inducer L-rhamnose needed to induce expression of the recombinant polypeptide. (Office Action dated April 28, 2009, page 10). Applicants strongly disagree.

Nothing in the references teaches or suggests the substitution suggested by the Examiner or that such a substitution would lead to a reduced amount of L-rhamnose needed. Rather Moralejo teaches that only the RhaB leader region functions as a promoter with no significant activity detected from RhaA and RhaD constructions (Moralejo, abstract). Furthermore, the isomerase RhaA catalyzes the reaction of L-rhamnose to L-rhamnulose and the rhamnulose kinase RhaB catalyzes the reaction of L-rhamnulose to rhamnulose-1-phosphate. The isomerase RhaA and the rhamnulose kinase RhaB relate to different parts of the pathway and they are totally different enzymes which catalyze totally different reactions. Further Wilms teaches that a much higher concentration of L-rhamnose than that claimed is needed for sufficient expression, as explained above. One skilled in the art would not substitute inactivation of a L-rhamnulose kinase with inactivation of a L-rhamnose isomerase. Furthermore, neither Wilms nor Moralejo teach or suggest the desirability of such a substitution. Neither Wilms nor Moralejo teach or suggest that a method with such a substitution would lead to reduced amount of L-rhamnose needed. Neither Wilms nor Moralejo teach or suggest that a method with such a substitution would work with the L-rhamnose concentration as presently claimed, actually Wilms teaches the contrary. *In re Mills*, 916 F.2d 680, 682, 16 USPQ2d 1430 (Fed. Cir. 1990); *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992) (the mere fact that a reference may be modified to reflect features of the claimed invention does not make the modification, and hence the claimed invention, obvious unless the prior art suggested the desirability of such modification); *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986) (a reasonable expectation of success must be established for a proposed combination of references to render claims *prima facie* obvious.); *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991) (the reasonable expectation of success must be found

in the prior art). Thus, Wilms and Moralejo are not combinable and do not render the claims obvious for these additional reasons.

Assuming *arguendo* that the references were combinable, the combination would still not arrive at the claimed invention. For example, the substitution of the rhamnulose kinase RhaB for the isomerase RhaA of Moralejo in the method of Wilms would still not arrive at the present method with the concentration of L-rhamnose as claimed, since the method of Wilms being modified by this substitution would still require the high amount of L-rhamnose taught in the method of Wilms.

Assuming *arguendo* that the Examiner had established a *prima facie* case of obviousness, a *prima facie* case of obviousness is rebuttable by evidence that the claimed invention possesses unexpectedly advantageous or superior properties. *In re Papesch*, 315 F.2d 382 (CCPA 1963).

The present invention relates to an improved method for expressing nucleic acids in prokaryotic cells using the rhaBAD promoter where surprisingly small quantities of L-rhamnose give high expression levels (as explained above and in detail in the last response). Thus, the present method differs substantially from that described by Wilms. As such, even if the Examiner had established that the claims are *prima facie* obvious over the combination of Wilms and Moralejo, this *prima facie* case would be successfully rebutted by the unexpected and superior results achieved from using the process as claimed with a host cell deficient with regard to L-rhamnose isomerase when compared with the system of Wilms with a totally different enzyme, L-rhamnulose kinase, and the claimed concentration of L-rhamnose. (See also specification at page 6, lines 7-43, for further advantages of the present method; see detailed explanations in the Amendment and Reply of December 22, 2008).

In sum, because Wilms and Moralejo, alone or in combination, do not teach all the claim limitations, because the reactions taught by Wilms and Moralejo are different from the claimed process, because the references lead away from the substitution suggestion by the Examiner, because Wilms and Moralejo are not combinable, and because assuming *arguendo* they were combinable there is no expectation of success, a *prima facie* case of obviousness has not been established. Furthermore, assuming *arguendo* that a *prima facie* case of obviousness had been established, the unexpected results successfully rebut any finding of *prima facie* obviousness. *See In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988) (holding that if an independent claim is

nonobvious then any claim dependent therefrom is nonobvious). In light of the amendment, the reasons of record and the above reasons, reconsideration and withdrawal of the rejection is respectfully requested.

Wilms in view of Moralejo and Israelsen

Claim 12 stands rejected as being obvious under 35 U.S.C. § 103(a) over Wilms in view of Moralejo and Israelsen *et al.* (U.S. Patent No. 5,837,509; hereinafter "Israelsen"). Applicants respectfully disagree. However, in order to expedite prosecution, the claims have been amended without disclaimer or prejudice and recite the concentration of the L-rhamnose in the medium. Applicants respectfully urge reconsideration of the rejection for the reasons of record and for the following reasons.

The explanations provided above under obviousness rejection over Wilms and Moralejo are equally applicable to this rejection and are incorporated herein in their entirety.

In light of the amendments and explanations above, the rejection as to Wilms and Moralejo is believed to be rendered moot. Israelsen is relied on for allegedly teaching the proteins recited in claim 12. However, Israelsen does not remedy the deficiencies of Wilms and Moralejo. For example, as explained above, Wilms and Moralejo, alone or in combination, do not teach or suggest a concentration of L-rhamnose in the medium from 0.01 g/l to 0.5 g/l as now claimed in claim 1 and the claims dependent therefrom including claim 12. Since Israelsen is relied on for teaching the protein recited in claim 12, Israelsen also does not teach or suggest the claimed method with the concentration of L-rhamnose as claimed. Accordingly, Wilms, Moralejo, and Israelsen, alone or in combination, do not teach or suggest all the claim limitations, and as a *prima facie* case of obviousness has not been established. Reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

For at least the above reasons, Applicants respectfully request withdrawal of the rejections and allowance of the claims. If any outstanding issues remain, the Examiner is invited to telephone the undersigned at the number given below.

Accompanying this response is a Request for Continued Examination and a petition for a one-month extension of time to respond to the Office Action mailed April 28, 2009 with the

required fee authorization. No further fee is believed due. However, if any additional fee is due, the Director is hereby authorized to charge our Deposit Account No. 03-2775, under Order No. 12810-00091-US from which the undersigned is authorized to draw.

Respectfully submitted,

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